# T lymphoid cells in primary syphilis

# Quantitative studies

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SUMMARY In previous studies to assess the role of cell-mediated immunity in *Treponema* pallidum infection investigations of delayed type skin hypersensitivity tests, lymphocyte transformation test, leucocyte migration inhibition tests, and histological studies of the reticulo-endothelial system have been performed.

In this study, the numbers of T (thymus-dependent) lymphoid cells in 10 patients with primary syphilis (eight untreated) were estimated by the rosette technique. Results indicate a significant decrease in the number of T lymphoid cells in patients with primary syphilis.

#### Introduction

The results of studies to assess the role of cell-mediated immunity in *Treponema pallidum* infection are inconsistent and therefore difficult to interpret, although the general impression that cell-mediated immunity has a protective function in syphilis—but is somehow adversely affected during the early and infectious stages of the disease—still remains.

Studies of delayed type skin hypersensitivity towards *T pallidum* antigens<sup>1</sup> indicate that a specific anergy to antigens of *T pallidum* exists in the primary and secondary stage, as well as in general paralysis of the insane, without impairment of responses to other unrelated antigens.

The lymphocyte transformation test in human and experimental rabbit syphilis has been studied,  $^{2\cdot16}$  but the results are conflicting; in some studies, a normal lymphoblastic response to T-cell mitogen phytohaemagglutinin (PHA), but reduced proliferation to treponemal antigens without suppression by syphilitic sera, was observed,  $^{7\cdot8}$  whereas in other studies a diminished lymphoblastic response to T-cell mitogens (concanavalin-A (con-A) and PHA) but normal proliferation to T pallidum antigens was found, although the latter could be suppressed by syphilitic sera.  $^{3\cdot9\cdot13\cdot15}$ 

The results of studies using the leucocyte migration inhibition test  $^{10}$  17-20 are more consistent and agree with the results of delayed type skin hypersensitivity tests, indicating a diminished cell-mediated immunity towards T pallidum in primary and secondary syphilis.

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Investigations of the histological appearance of lymphoid tissues in syphilis have shown that thymus-dependent areas are adversely affected in early acquired syphilis and in congenital syphilis.<sup>21</sup> <sup>22</sup>

Another method of assessing the cellular limb of immunity in syphilis is by determining the number of circulating T lymphoid cells in relation to the general distribution of lymphoid cells in patients with syphilis. In the present study, the number of circulating T lymphoid cells was estimated in 10 patients with primary syphilis by the rosette technique<sup>23</sup> and compared with that in a healthy control group.

# Patients and methods

STUDY GROUP

The patients included in this study were referred from the outpatient department of our hospital. A total of 10 patients was studied (the same as in a previous study<sup>24</sup>).

All patients were male, and a diagnosis of primary syphilis was based on the existence of an indurated ulcer from which *T pallidum* could be demonstrated by darkfield microscopy and on a positive result to a treponemal serological test at the initial or at a subsequent examination.

Eight of the patients were untreated at the time of investigation whereas two had been treated for less than one week.

## **HEALTHY CONTROLS**

Ten healthy staff members served as controls for the quantitative determination of T lymphoid cells. All were male and of the same age range as the study group.

# ISOLATION OF LYMPHOID CELLS

Lymphoid cell separation was carried out as described previously<sup>24</sup> according to the method of Böyum,<sup>25</sup> with some minor modifications as described below.

Ten millilitres of peripheral blood was collected aseptically by venepuncture in Erlenmayer bats containing 10 glass beads and shaken for 10 minutes for defibrination. To this was added 30 ml of phosphate-buffered saline (PBS). Portions of 10 ml of this suspension were layered on top of portions of 2.5 ml Ficoll-Isopaque mixture and centrifuged at  $400 \times g$  for 10 minutes. The resulting lymphoid-cellrich layer between the Ficoll-Isopaque and the supernatant was aspirated and washed three times in PBS. After each washing, the lymphoid-cell suspension was centrifuged at  $200 \times g$  for five minutes. The lymphoid-cell-rich layer was composed of about 90-95% lymphoid cells and 5-10% monocytes and neutrophils. Viability of the isolated cells was over 98% as determined by trypan blue exclusion.

# DETERMINATION OF T LYMPHOID CELLS

Isolated lymphoid cells  $(1 \times 10^6)$  were resuspended in 0·3 ml minimal essential medium (MEM); to this was added 0·3 ml of fresh sheep red blood cell (SRBC) suspension containing a total of  $50 \times 10^6$  SRBC: this suspension of SRBC had previously been washed four times in MEM, each time at  $200 \times g$  for 10 minutes. The whole suspension of lymphoid cells and SRBC was centrifuged at  $200 \times g$  for five minutes and then incubated for one hour at 4°C. It was resuspended carefully. Only those lymphoid cells which had attracted at least three SRBC to form a rosette were counted (by light microscopy); these were T lymphoid cells and their number was expressed as a percentage of the total lymphoid cells in suspension.

# PERIPHERAL BLOOD COUNTS

Total peripheral leucocyte counts and their differentiation were estimated to relate percentage figures to the absolute numbers of lymphoid cells.

# STATISTICAL METHOD

The non-parametric Wilcoxon's test, <sup>26</sup> with the usual correction for ties and the normal approximation to obtain the P value, was used for statistical analysis of the numbers of T lymphoid cells in the study group and in controls.

#### **MATERIALS**

Isopaque (Natrii N-, ethyl-3,5-diacetamido-2.4.6-trijodbenzoas) solution: 20 ml Isopaque (Nyegaard

and Co, Oslo, Norway) was diluted with 25 ml distilled water.

Isopaque-Ficoll mixture: 10 parts Isopaque solution were mixed with 24 parts of Ficoll (9% in distilled water) (Pharmacia, Uppsala, Sweden).

Phosphate-buffered saline (PBS), pH 7·4:8·2g NaCl +1·6 g Na<sub>2</sub>HPO<sub>4</sub>.2H<sub>2</sub>O+0·2 g NaH<sub>2</sub>PO<sub>4</sub>.2H<sub>2</sub>O dissolved in I litre distilled water.

Minimal essential medium (MEM): A modified Eagle's solution containing 2 g/l sodium bicarbonate without glutamine (Flow Laboratories, Irvine, Scotland).

Sheep red blood cells (SRBC): Fresh sheep red blood cell suspension in Alsevers medium (Central Laboratory of the Red Cross Blood Transfusion Services, Amsterdam, the Netherlands).

Trypan blue: 0.05% in PBS.

## Results

The results of T lymphoid cell determination in 10 patients with primary syphilis are given in the Table, together with those in the 10 healthy controls. Absolute numbers of lymphoid cells in the study group did not differ from normal (mean:  $2 \cdot 35 \times 10^9/l$ ; range  $1 \cdot 66 \cdot 2 \cdot 95 \times 10^9/l$ ), and statistical analysis was performed on percentage figures.

TABLE Percentages of T lymphoid cells in 10 patients with primary syphilis and in 10 healthy controls

Case No	T cells (%)	Control No	T cells (%)
2	45	1	62
3	63	2	63
4	50	3	62
5	53	4	69
6	50	5	61 · 5
7	51	6	65.5
8	63	7	59
9*	53	8	54.5
11	49	9	64
12*	53	10	65
Range	45-63	Range	54 · 5 - 69 · 0
Median	52.0	Median	62.5

<sup>\*</sup> Patients who had been treated for less than a week Standardised W = -2.9593

P value = 0.0035

The exact values of standardised W and two-sided tail probability, after Wilcoxon's test had been applied to the percentages of T lymphoid cells, are given in the Table. The number of T lymphoid cells in the study group and the control group was significantly different (P = 0.0035), with a difference in medians of -10.5%, indicating a decrease in T lymphoid cells in primary syphilis.

# Discussion

In this study, the evidence suggests that the number of T lymphoid cells in patients with primary syphilis is decreased; only two out of 10 had normal percentages (cases 3 and 8). This decrease in the number of T cells is associated with an increase in IgG-bearing lymphoid cells found in the same patients. <sup>24</sup> Thus the selective depletion of T lymphoid cells in the paracortical areas of lymph nodes from patients with primary syphilis<sup>21</sup> seems not to be accompanied by an increase in the number of circulating T cells. It is uncertain whether or not the decrease in circulating T cells is another effect of the serum factor(s) that depress the lymphoblastic response to T pallidum antigens of isolated lymphoid cells, especially enriched T cell populations. <sup>13</sup>

The exact nature of the suppressing factor(s) in syphilitic sera is still unknown; they may be of treponemal origin (soluble antigens) or there may be a shift in the homeostasis of soluble mediators of immune regulation.<sup>27</sup> In this respect it is interesting that the production of lymphocyte mitogenic factor was impaired in lymph node cells of rabbits infected with *T pallidum*.<sup>16</sup>

Decreased concentrations of circulating T cells in primary syphilis is certainly not disease-specific; these have been found in a wide variety of diseases, infectious as well as non-infectious. <sup>28</sup> This study provides no information on the exact nature of the decrease in T cells—whether there is a selective depletion of a subclass of T cells or whether the decrease is the result of an interaction of serum factor(s) on the physicochemical binding of T cells and SRBC with or without impairment of immune function. Studies are at present being directed towards the elucidation of some of these possibilities.

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